

The University of Chicago

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TO PIGMENTATION AND TO TESTIS
DESCENT IN THE OPOSSUM AND
GROUND SQUIRREL

A DISSERTATION SUBMITTED TO THE FACULTY OF THE
DIVISION OF THE BIOLOGICAL SCIENCES IN CANDIDACY
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF ZOOLOGY

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THE RELATION OF SEX HORMONES TO PIGMENTATION AND TO TESTIS DESCENT IN THE OPOSSUM AND GROUND SQUIRREL¹

MIRIAM POSNER FINKEL

Hull Zoological Laboratory, University of Chicago, Illinois

SEVEN TEXT FIGURES AND THREE PLATES (TEN FIGURES)

INTRODUCTION

The deep-lying pigment associated with the reproductive tract in marsupials has been noted by a number of investigators. The black color of the gubernacular apparatus of the immature male, of the parietal tunica vaginalis of the adult male, and of the homologous gubernacular tissue of the female Virginia opossum was noticed by C. R. Moore and his associates in the course of investigations dealing with sex differentiation and its experimental modification conducted in this laboratory. Earlier Klaatsch (1890) mentioned the presence of pigment cells in the inguinal ligament of young *Didelphys*, and van den Broek ('10) described the black tunica vaginalis propria in adult *Didelphys*, *Dasyurus*, *Petaurus*, and other *Phalangeridae*. Frankl ('00) found pigment also in the epididymis of *Petaurus* and in the entire vaginal process and mesorchium of *Halmaturus*. The pigmented condition of the male and female gubernacular apparatus in 20- and 50-day-old pouch young of *Didelphys virginiana* was described by Burns ('39 c, d). Moore ('41) illustrated the black parietal tunica vaginalis of the adult male and the pigmented structure in a 31-day female. *Trichosurus vulpecula*, the Australian opossum, likewise possesses pigment in the parietal layer of the processus vaginalis (Bolliger and Carroddus, '39).

¹ This investigation has been aided by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

Several questions regarding this pigmentation immediately suggest themselves: (1) What is the embryonic origin of the melanophores responsible for this non-integumentary pigmentation? (2) Is this a characteristic of adaptive value, executing a definite function in the physiology of the animal, or merely an incidental trait common to many marsupials? (3) Since the pigmentation is associated with the reproductive tract, is it under the control of sex hormones? The present study was initially undertaken to answer, as far as possible, the preceding questions. It was soon discovered that a knowledge of the morphogenesis of the gubernacular apparatus in both male and female opossums is essential to an adequate understanding of the pigment and its distribution. Consequently, observations on the normal development and possible hormonal modification of this composite structure are included in this report.

As an adjunct to the opossum study, the histology, periodicity, and hormonal control of the scrotal pigmentation of the ground squirrel were investigated. *Citellus tridecemlineatus* has been an experimental subject in this laboratory since 1931. Though no critical analysis was made of the peculiar cyclical coloration of the scrotum, it was early recognized that pigmentation is associated with reproductive activity and that animals not in breeding condition possess colorless scrota. Wells ('35) described the annual rhythm of pigmentation as being similar to that of the accessory reproductive glands, and found that the degree of color served as a useful criterion of reproductive condition (Wells, '36 a; Zalesky and Wells, '37; Wells and Zalesky, '40). Additional indication of its accessory-like behavior is given by Baker and Johnson ('36), who produced scrotal pigmentation in immature male ground squirrels by injecting human chorionic gonadotropin (Antuitrin-S).

MATERIALS AND METHODS

Opossum

Two hundred and twenty-two male and female opossums provided the material for this study. Of the series, 130 were

from the slide collection of Prof. Carl R. Moore and 3 from that of Dr. David Rubin. All are Bouin-fixed, Harris hematoxylin-stained, 10 micra serial sections. Additional material was sectioned at 4 to 10 micra and stained with aqueous alum cochineal. Gross examination alone was made of some fifty individuals. The distribution of sexes, the hormonal treatments, and the age ranges are summarized in table 1.

TABLE 1
Distribution of experimental opossums.

TREATMENT	MALE		FEMALE	
	Number of animals ^a	Age at autopsy ^b	Number of animals ^a	Age at autopsy ^b
Normal	93	Day 5-100. Adult	33	Day 7-198. Adult
Androgen	18	Day 16-100.	16	Day 16-168. Adult
Estrogen	11	Day 12- 88.	11	Day 17- 79. Adult
Gonadotropin	8	Day 15-100. Adult	8	Day 15-125. Adult
Gonadectomy	7	Day 52-122.	12	Day 52-130. Adult

^a Five normal, sexually indifferent individuals (four day 1 and one day 3) complete the list.

^b The age of animals of unknown delivery date was estimated on the basis of snout-rump length, head length, and body weight in comparison with known-aged young (Moore and Bodian, '40).

Experimental procedure involved treatment with androgenic, estrogenic, and gonadotropic hormones, and gonadectomy.² (1) Male hormone in the form of a testosterone or testosterone propionate, lanolin-base ointment was administered by inunction to pouch young as early as day 3. The ease of cutaneous absorption of sex hormones has been demonstrated by Moore, Lamar, and Beck ('38). These animals received subcutaneous injections of testosterone propionate

² Grateful acknowledgment is made to Dr. Gregory Stragnell, Shering Corporation, for testosterone and testosterone propionate ointments and for estradiol benzoate (Progynon B); to Dr. George Cartland, Upjohn Company, for equine gonadotropin (Gonadogen); to Dr. Donald Wonder, Cutter Laboratories, for equine gonadotropin (Gonadin); and to Dr. Oliver Kamm, Parke Davis and Company, for estrone (Theelin).

when carried past day 15, as did others placed under treatment on day 10 or later. Pouch young received 0.25 mg. of the hormone per injection. (2) Estradiol ointment, applied cutaneously, was the method of female hormone administration in the majority of cases. A few animals received subcutaneous injections of 25 I.U. (2.5 μ g.) of estrone (Theelin) each day of treatment. One hundred and twenty-five I.U. (12.5 μ g.) per day of estradiol benzoate (Progynon B) were given to one male and one female which were autopsied on days 17 and 19 respectively; two males killed on days 83 and 88 received 20 I.U. of Progynon B each day of treatment. (3) The gonadotropic principle of pregnant mare serum (5 r.u. of Gonadin or 1 r.u. of Gonadogen) was given by daily subcutaneous injections. (4) Gonadectomy between days 20 and 40, a time during which the animal once removed cannot successfully reattach to a nipple, was performed by Professor Moore, using the technique he developed for operating on pouch young (Moore, '41 a, '43). Four males were castrated at that stage of testis descent in which the gonad is in the scrotal cavity though not deep within its fundus (estimated day 72). This was accomplished by removal of the testis through a small, lateral, scrotal incision. A ventral rather than a dorsal approach in ovariectomizing adult females was found to be more successful.

No reasonable method presented itself for determining the total number of melanophores in the male since in the developmental stages pigment cells occur throughout the parietal tunica vaginalis and a portion of the gubernacular apparatus. For purposes of comparison counts were made in the region of greatest melanophore density (the parietal tunica vaginalis just past the point where it becomes continuous with the gubernacular apparatus) in animals 100 days or younger. Melanophores were counted in the gubernaculum proper of animals which were 107 and 122 days old. With the aid of an ocular which divided the field into squares all the melanophores located within a 205×205 micra area of a 10-micra section were counted at a magnification of 440. This method

leaves much to be desired; e.g., whether or not to consider a small pigmented process as a "cell" becomes an important question when the total number in the area is small. Also stretching of the tissue at the moment of fixation alters melanophore concentration in such a small region. Despite these and other faults this technique was considered sufficiently accurate and valuable to warrant its employment.

An attempt was made to classify the male melanophores on the basis of degree of pigmentation and extent of cell processes. A 3 x 3 table was constructed with the ordinate characters: (1) relatively few, light brown melanin granules, (2) medium density of granules of brown-black color, and (3) heavy pigmentation. The abscissal categories were: (1) no visible processes, (2) relatively short or medium processes, and (3) long, slender protoplasmic extensions. Any melanophore sufficiently complete in the examined section was entered in this table and was given a numerical value which depended on the product of the marginal weights. Thus, a light-brown melanophore with short processes would have a weight of 2, and a brown-black melanophore with long, slender processes would have a weight of 6. For each animal the average melanophore type was expressed by an index number which was the mean of the weighted entries and which had a possible range from 1 (light color and no processes) to 9 (black color and long processes). Several criticisms of this method of classification can be made, but no more adequate procedure suggested itself. For example, it was impossible to diagnose many cells because of their partial presence in a 10-micra section. Though correlation between degree of color and extent of processes is by no means complete, sufficient association is evident to suggest that the chosen qualities have some relationship.

Estimation of total melanophore number in the female was possible because of the discreteness and relatively small size of the pigment-containing structure, the so-called round-body. The pigment cells in one-tenth, on the average, of the whole organ were counted at a magnification of 550; i.e., if the struc-

ture extended through 210 sections, the melanophores were counted in every twenty-first section, totaled, and multiplied by ten. Though this method gives a reasonably accurate and legitimate estimate, several sources of error are unavoidable. (1) A melanophore was considered to be present in a given section even if it was represented by a single process. This would tend to make the total count too high. (2) Lightly pigmented cells were easily missed because of the lack of contrast offered in hematoxylin-stained preparations. (3) It was extremely difficult in heavily pigmented regions to avoid counting some cells more than once and some not at all.

Relative weights of the round-bodies were determined by projecting each section at a 48.5 magnification on paper of uniform thickness, cutting out the traced sections, and weighing them.

Differences between normal and treated animals were examined for statistical significance by making use of comparisons between appropriate pairs. Since melanophore number and round-body weight are functions of growth, logarithms were used instead of the absolute values. The differences were analyzed by "Student" 's *t*-test, and the results are here expressed in terms of *P* ("Student", '25). Values of *P* less than 0.05 are customarily considered significant; values less than 0.01 are very significant.

Ground squirrel

The male ground squirrels used in this study were collected 20 to 30 miles south and southwest of Chicago. Those designated "field animals" were collected between April 5 and October 9, 1943, and were autopsied or used experimentally within a few days of capture. "Laboratory animals" are those which had been taken in the field between May 8 and September 1, 1942 and had wintered in a heated, sky-lit, animal room in Hull Laboratory. The two age categories of Wells ('35) and others have been employed; animals are referred to as "adult" if they have produced spermatozoa and "immature" if they have not. In spring and early summer

general appearance and body weight are sufficient to indicate whether or not an individual was born in the previous breeding season. A useful criterion is the fact that the glans penis can be easily everted from the prepuce by manual pressure in adults but not in young. Since the body weight of first-season males approaches or even exceeds that of adults in autumn, gross examination of the testes was used to give an indication of the approximate age of the animal near the end of the collecting season. At that time the gonad of the adult was shrunken, greyish-white, and unmistakably retrogressed; that of the immature was small, pink, and firm.

A majority of the 195 animals used served to establish the normal relationships among degree of scrotal pigmentation, weight of gonads, and weight of accessories (seminal vesicles and prostate). Experimental treatments involved subcutaneous injection of either testosterone propionate or equine gonadotropin, and castration.

At autopsy the scrotum, the right side of the belly, and the left side of the back just below the last rib were carefully shaved, and samples of dorsal and ventral skin were taken. The scrotal skin was removed by a cut which started caudad to the anal aperture, proceeded into the groin on either side, and extended medio-anteriorly to the base of the penis. The excised tissue was fixed after being stretched and pinned in a waxed tray. Weights were taken of testes, seminal vesicles, and prostates before preservation in Bouin's fluid. Tissues were sectioned at 4 to 10 micra and stained with aqueous alum cochineal, Harris' hematoxylin, or Mallory's connective tissue stain as modified by Heidenhain.

The degree of scrotal pigmentation of adult animals was recorded at the time of autopsy on an arbitrary scale ranging from 0 (no pigment) to 10 (extreme black color). Laboratory animals which were observed but not killed were recorded in the same manner; pigmentary changes could thus be followed in the same group of animals. A more accurate and rigorous classification of the isolated scrota in alcohol was possible. To differentiate this scale from the preceding, "a"

(0.0) designates lack of color and "f" (1.5) intense black pigmentation. (Numerical values were assigned to facilitate averaging and graphing.) Comparison shows such close agreement between the autopsy and alcohol classifications that transposition of observations of living animals from the "0-10" to the "a-f" scale is justifiable.

The scrotal pigmentation and general body color of normal immature animals appear so different from those of adults that an additional pigmentary series was devised. In this independent, juvenile scale, which runs from 0 to 8, "j0" indicates absence of color and "j8" the darkest scrota observed.

THE OPOSSUM GUBERNACULAR APPARATUS

By "gubernacular apparatus" is meant that system of mesenteries and ligaments which directs the descent of the testis in the fetal or young male and which, in the female, persists as the round and ovarian ligaments. The degree of its retention in the adult male mammal is variable. The contents of the scrotal cavity of an adult opossum are shown in figure 1, in which the heavily pigmented parietal tunica vaginalis is illustrated as opened and stretched out. The broad mesorchium is seen extending from the lateral surface of the testis to the epididymis. That portion of the epididymis above the emergence of the spermatic cord lies free, whereas the tail and inferior half of its body are firmly attached to the parietal tunica vaginalis by a slender, triangular mesentery. There is no gubernaculum, *per se*, in the adult male opossum. This agrees with Frankl's description ('00) of two other species of *Didelphys*. Van den Broek ('10) mentions a thick band of connective tissue running between the scrotal skin and the testis in adult *Didelphys* (sp.); this corresponds to nothing seen here in *Didelphys virginiana*.

The ligamentum rotundum and ligamentum ovarii proprium of the adult female opossum differ significantly from those of other mammals in only one respect. At the juncture of these two mesenteries, lying more or less close to the uterus, is a small, pigmented, discrete mass of tissue. From it a delicate,

darkened thread extends along the ventral border of the round ligament. Burns ('39 d), describing a normal, 50-day female opossum, refers to the free-lying "head" and attenuated "handle or stalk" of this structure. He implies that it alone represents the female gubernaculum. Since it is known

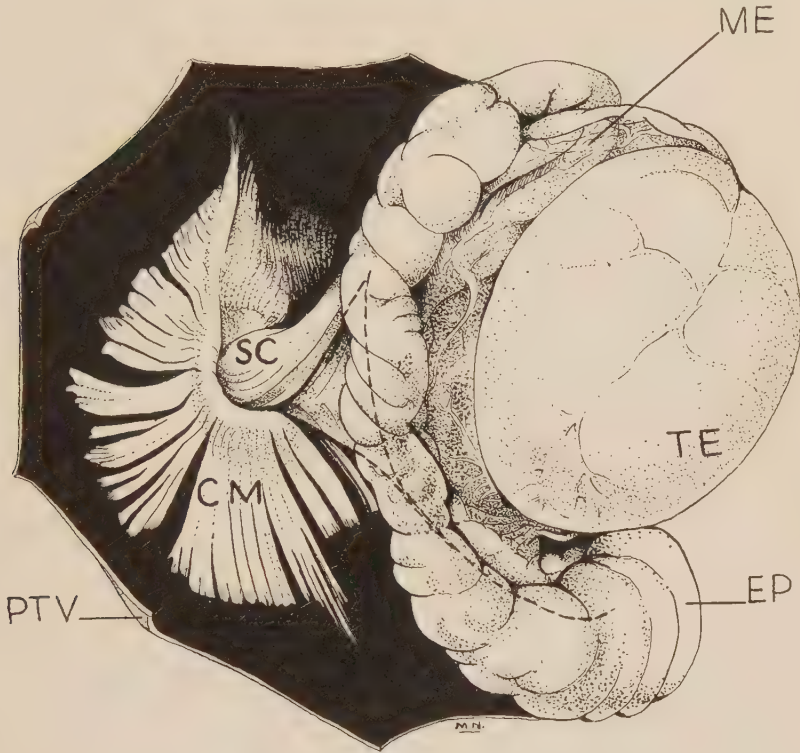


Fig. 1 Drawing of scrotal contents of adult normal male opossum. CM, cremasteric muscle; EP, tail of epididymis; ME, mesorchium; PTV, parietal tunica vaginalis; SC, spermatic cord; TE, testis.

that the round and ovarian ligaments are homologous to portions of the male apparatus, an additional name is required. In this report "round-body" denotes the pigmented organ of the gubernacular apparatus of the female opossum.

Careful dissection is required to locate the very small, obscure round-body in the adult. It varies in shape from slightly

oval to elongate, in length from 1 to 3 mm., and in color from pale grey to black. Since it is extremely conspicuous in immature individuals, it is illustrated in a normal female estimated to be 85 days of age (fig. 2). The proportionately greater growth of the whole tract as compared to that of the round-body accounts for its relative imperceptibility in older animals.

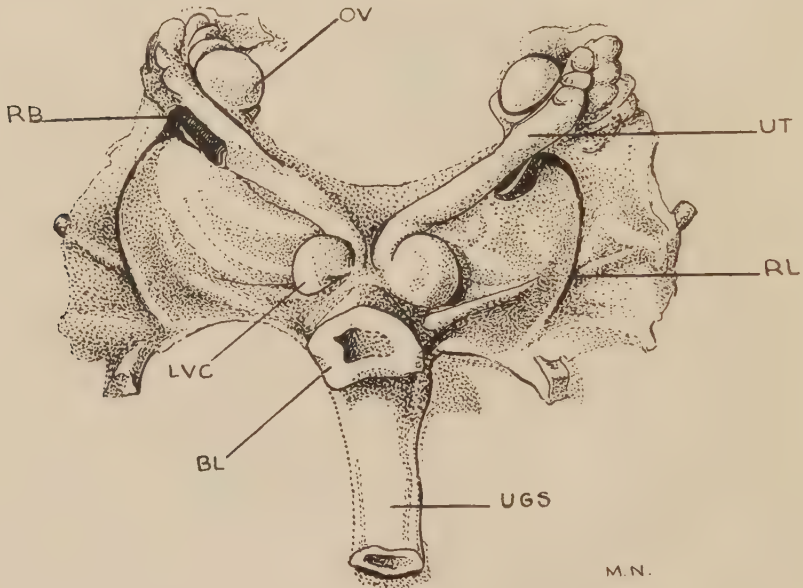


Fig. 2 Drawing of reproductive tract of 85-day normal female opossum. BL, bladder; LVC, lateral vaginal canal; OV, ovary; RB, round-body; RL, round ligament; UGS, urogenital sinus; UT, uterus.

The parietal tunica vaginalis of the male and the round-body of the female owe their color to large, dendritic, frequently anastomosing melanophores. The pigment cells illustrated in figure 8 were photographed from an unstained whole mount of the tunica vaginalis propria and underlying tunica vaginalis communis. The large, oval nuclei (average size 13.7×8.5 micra) appear as light areas within the dark cytoplasm. The melanin granules range in shape from small spheres to rods, in color from pale yellow-brown to black, and in longest di-

mension from 0.2 to 1.2 micra. The length and number of protoplasmic processes is variable; one cell possessing three major extensions measured over 226 micra from tip to tip.

No deep-lying pigment other than that associated with the reproductive tract has been observed in the opossum. With the exception of that of the eye, all other pigmentation occurs in the integument, appearing primarily in the hair follicles at the time the colored portion of the hair is being formed. Dermal melanophores pervade the black spots of the ear and of the scrotal and marsupial skin, and they appear elsewhere in occasional body spots (e.g., on the chest). The back of the fore-foot, sole of the hind-foot, and dorsal root of the tail owe their color to dermal melanophores. Very sparse epidermal pigmentation likewise occurs in the sole of the hind-foot. The only region containing epidermal pigmentation to any marked degree is the dark band encircling the tail a short distance from its base.

Normal development

The "indifferent" period. Five pouch young prior to their gonadal sexual differentiation were available for study. Morgan ('43) states that ovarian development is detectable at 3 to 5 days and that the 5-day gonad is clearly perceptible as male or female. No other criterion for ascertaining the sex of such young animals has been presented.

Four animals were killed on day 1, and these possess recognizable gubernacular rudiments. However, gubernacular development has proceeded along two different lines with two individuals in one category and the other two in a second category. This dimorphism indicates that the sex of the individual might be distinguishable prior to gonad differentiation. In the first two individuals a mass of proliferating cells located just below the ventral peritoneum lateral to the urinary bladder is pierced by a small coelomic evagination. A cord of cells extends upward from the ventral dermis and approaches that growing down from the peritoneum (fig. 9). According to the terminology of Felix ('12), the proliferation

from the peritoneum is the chorda gubernaculi in the narrow sense (anlage of the pars muscularis of the gubernacular apparatus), its incipient cavity is the saccus vaginalis, and the cord of tissue extending from the ventral dermis is the ligamentum scroti (the future pars scrotalis of the gubernacular apparatus). In the second two individuals the ligamentum scroti is lacking and the chorda gubernaculi is less extensive. From the lateral margin of the very small saccus vaginalis a knob-like mass of cells projects into the coelomic cavity. This is the inguinal crest, which unites with the inguinal fold (a lateral proliferation growing down from the tubar portion of the mesonephros) plus the head of the chorda gubernaculi (fig. 10). The condition of the gubernacular apparatus in animals in which sex can be definitely diagnosed (day 5 and later) suggests that the individuals in the first category are males and that those of the second are females.

By the use of the presence of the ligamentum scroti and the absence of the inguinal crest, inguinal fold, and chorda gubernacular "head" in 1-day opossums as male criteria, the fifth opossum, a 3-day animal, can be readily diagnosed as a male. An inguinal crest and inguinal fold are present and joined, but both are smaller and structurally different from those seen in the 1-day "female".

The male gubernacular apparatus. By day 5 the saccus vaginalis has penetrated the ventral body wall until it extends to a point midway between the ventral integument and the peritoneum. The gubernacular apparatus consists of (1) the ligamentum scroti, anchored in the dermis of the already recognizable scrotum, (2) the chorda gubernaculi, continuous posteriorly with the former and anteriorly with (3) the pars inguinalis, derived from the fused inguinal fold and inguinal crest. Although McCrady ('38) did not detect scrotal rudiments until an age of 10 to 11 days, they have appeared histologically by day 5. Figure 11 illustrates the condition in an 8-day male. A few sections posteriorly the chorda gubernaculi fuses with the lateral wall of the saccus vaginalis.

The pars mesonephridica, which forms in the tubar portion of the mesonephros and is continuous with the pars inguinalis, makes its appearance by day 10. Anteriorly it unites with the mesorchium, or pars mesorchica of the gubernacular apparatus. Thus a continuous band of tissue lies between the testis and the scrotum. By day 14 the wolffian duct, since it is closely associated with the gubernacular apparatus through the pars mesonephridica, is pulled toward the mouth of the saccus vaginalis (the inguinal canal) as general body growth continues.

Melanophores appear for the first time in two out of the three 15-day normals examined. Occasional cells are found lateral to the inguinal opening, in the parietal tunica vaginalis, in the caudal portion of the chorda gubernaculi, and in the ligamentum scroti. Similar distribution of somewhat more numerous pigment cells was noted in four 16-day individuals.

On day 24 the testis lies just without the inguinal canal whereas the bulk of the wolffian duct is contained within the saccus vaginalis. Seven days later the testis is enclosed within the saccus. The chorda gubernaculi attaches to the medio-dorsal wall of the saccus vaginalis cavity, which at this time has further penetrated the ventral body wall (fig. 12). Though most concentrated in the chorda gubernaculi and in the pars scrotalis (the former ligamentum scroti), which is beginning to enter the scrotal swelling, scattered pigment cells are found in the tunica vaginalis and surrounding connective tissue. Melanophores are not restricted to the peritoneal covering of the gubernaculum and the lining of the saccus vaginalis, as suggested by Burns ('39 d), but are distributed throughout the tissue of the former and into and beyond the tunica vaginalis communis of the latter.

Continued medio-ventral growth of the saccus vaginalis into the pars scrotalis causes the testes to be drawn toward one another so that they almost meet medially close to the ventral integument and scrotal swelling by day 52. They are separated only by a band of connective tissue between the two sacci (the anlage of the septum scroti). The inguinal

canal is closed. Contrary to the observation of Burns ('39 d), who states that the testis is directly attached to the scrotal wall by the contracted gubernaculum in 50-day-old normal males, the mesorchium joins the testis to the epididymis, which in turn is attached to the parietal tunica vaginalis by the compound ligament. The tissue surrounding the saccus vaginalis is erroneously referred to as "scrotal wall" since the scrotal swelling at this age has been penetrated by the saccus only at its most anterior limit. Figure 13 shows the gubernacular relationships near the caudal limits of the testis. The heavily pigmented chorda gubernaculi, which is continuous with the parietal tunica vaginalis, appears a few sections posteriorly. Continued migration of the saccus vaginalis into the pars scrotalis brings the testes partially within the scrotal swelling by day 74, and descent is completed by day 77.

The postero-ventral migration and growth of the saccus vaginalis is accomplished at the expense of the chorda gubernaculi, and the gubernaculum becomes progressively shorter by the reflection of its tissue into the wall of the saccus as penetration proceeds. At the completion of testis descent at 77 days a relatively small cone of cells remains to anchor the epididymis to the vaginal sac. The subsequent enlargement of the cavity associated with testicular and epididymal growth further decreases the ligament until the epididymis becomes firmly applied to the wall of the saccus vaginalis.

Though usually present at 15 days and regularly present after 16 days, pigmentation of the gubernaculum and parietal tunica vaginalis was entirely lacking in one 30-day and in one adult untreated animal. Two cases (day 52 and adult) were observed of unilateral absence of melanophores. No other gross anatomical or histological abnormality of these animals was detected either in the testis or in any part of the gubernacular apparatus or tunica vaginalis.

The female gubernacular apparatus. In the 1-day "female", it will be recalled, the gubernacular apparatus was represented by a minute saccus vaginalis and fused inguinal crest and inguinal fold. The round-body anlage was already evi-

dent as the knob-like portion of the chorda gubernaculi associated with the inguinal crest. By day 7 the remainder of the chorda gubernaculi appears as a band of tissue extending from the enlarged round-body to the microscopically discernible marsupial fold rudiment (fig. 14). In the human female this ligament joins the ligamentum labiale (homologous to the male ligamentum scroti) and thus forms a continuous band from the lateral uterine wall to the labia majora (Felix, '12). In the female opossum, on the other hand, a homologue of the ligamentum scroti does not appear, and all but the head of the ligamentum gubernaculi begins to retrogress by day 10 and is gone 2 days later. The round ligament of the adult, homologous to the chorda gubernaculi in the human according to Felix, is here seen to be composed of the pars inguinalis of the gubernacular apparatus, whereas the chorda gubernaculi remains only as the round-body. Mesonephric reduction and true pelvis formation have caused an apparent migration of the pars inguinalis (ligamentum rotundum) and round-body from their original ventro-lateral to a more dorso-lateral position by day 12 (fig. 15).

A few melanophores appeared in one 13-day normal female. One 14-day individual completely lacked pigmentation, but it was obvious in all others 16 days of age or older. Occurring first at the juncture of the mesentery and body wall, melanophores soon appear throughout the tissue of the round-body, in the ligamentum rotundum, and scattered below the parietal peritoneum. As previously noted in the male, it is not the peritoneal investment of the grossly pigmented structures that contains the pigment.

All components of the female gubernacular apparatus are present by day 24; a continuous cord extends from the ovary to the lateral body wall. The ligamentum ovarii proprium (pars mesorchica in the male) runs from the ovary to the broad ligament and inserts medio-dorsally in the tissue surrounding the müllerian duct — the pars mesonephridica. The round-body (chorda gubernaculi) lies at the juncture of the ligamentum rotundum (pars inguinalis) and the broad liga-

ment, which have their insertions close to one another on the body wall.

Subsequent development primarily involves changes due to caudal ovarian migration and general growth. Figure 16 illustrates the position of the round-body in a normal, 44-day female. The most heavily pigmented structure is the round-body itself, though many melanophores occur in the ligamentum rotundum. Occasional pigment cells at the juncture of the round ligament and the body wall mark the site of the transitory saccus vaginalis.

By day 85 uterine growth has carried the medial attachment of the ligamentum rotundum so far anterior that the mesentery now takes an antero-posterior rather than a medio-lateral course (fig. 2). More or less adult relationships thus are established with the insertion of the round ligament in the ventral body wall lateral to the urinary bladder.

Effect of treatments on the male

Number of melanophores. Melanophores were counted in normal, in hormonally treated, and in castrate animals by the method previously described in order to ascertain whether they are in any degree under gonadal control. The number of pigment cells per unit area at their most concentrated point in the parietal tunica vaginalis of ten normal animals from 15 to 100 days of age is graphed in figure 3. The first melanophores appear at day 15, their number rises sharply after day 24, and their peak is reached at day 52. Since individuals between days 52 and 83 were not available for study, it is not known whether or not a higher number is attained during this period. The reduction noted to day 83 in the material at hand is due to the further growth and penetration into the scrotal swelling of the saccus vaginalis. It will be recalled that the gonad, which was close to the scrotal swelling at day 52, is partially within it by day 74 and firmly anchored in its fundus by day 77. The consequent stretching of the parietal tunica results in a relative decrease in melanophore density.

Counts in normal and treated animals are given in table 2. Since in many instances normal individuals of the same age as the treated ones were not available for study, it became necessary to obtain estimates of the former by interpolation from the normal graph. Therefore too much emphasis must not be placed on any single comparative observation. The over-all picture, however, is indicative.

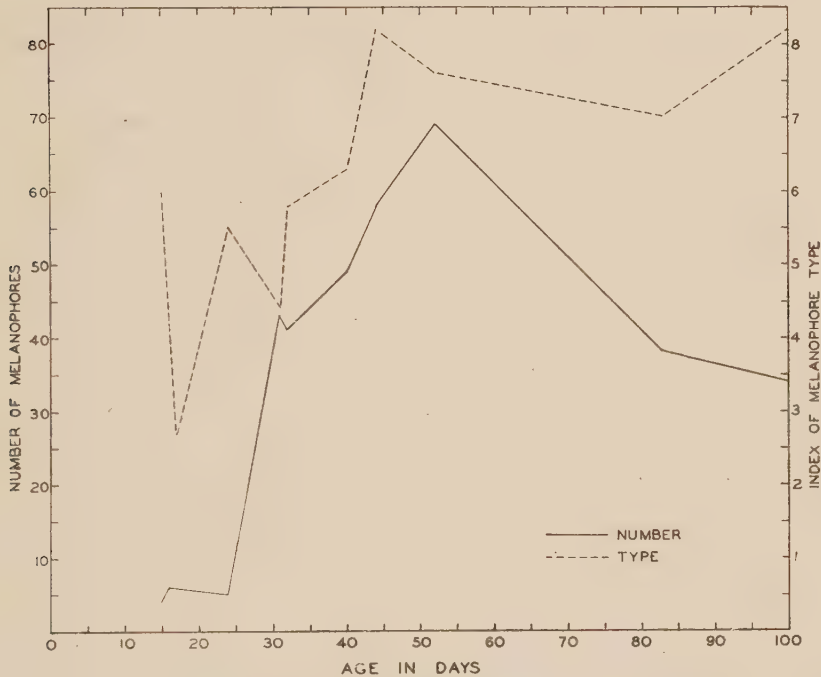


Fig. 3 The number per unit area and average type of melanophores in the parietal tunica vaginalis of normal male opossums.

Androgen treatment. Six of the eight androgen-treated animals possessed more melanophores than normals of comparable age. Since pigment has never been seen in the male prior to day 15, it is not surprising that testosterone treatment for 13 days before autopsy on day 16 results in no acceleration of melanophore differentiation. Analysis of the data from days 24 to 100 inclusive gives a P-value of 0.0532,

TABLE 2

Number of melanophores per unit area in the parietal tunica vaginalis.

AUTOPSY AGE IN DAYS	NUMBER OF MELANOPHORES		RATIO TREATED/ NORMAL	TREATMENT ^a
	Normal	Treated		
Androgen-treated				
16	6	6	1.00	3-15 TO
24	5	8	1.60	3-24 TO
30	(39) ^b	55	1.41	3-15 TO; 16-29 TP inj.
31	43	78	1.81	10-30 TP inj.
32	41	57	1.39	16-31 TP inj.
40	49	70	1.43	30-39 TP inj.
83	38	25	0.66	70-82 TP inj.
100	34	49	1.44	70-99 TP inj.
Estrogen-treated				
17	(6)	9	1.50	1-16 EsO
23	(5)	32	6.40	5-22 EsO
30	(39)	70	1.80	6-29 EsO
31	43	90	2.09	17-30 EsO
43	(56)	134	2.39	32-43 Th inj.
83	38	21	0.55	70-82 PB inj.
Gonadotropin-treated				
15	4	0 ^c	...	8-14 G inj.
17	(6)	0	...	10-16 Gg inj.
21	(5)	0	...	10-20 Gg inj.
30	(39)	19	0.49	15-29 G inj.
55	(66)	71	1.08	40-55 G inj.
Castration				
52	69	44	0.64	castrate day 20
55	(66)	97 ^c	1.47	castrate day 35
107 ^a	92 ^c	136	1.48	castrate day 72
122 ^d	34	84	2.47	castrate day 72

^a Abbreviations are as follows: TO = testosterone ointment, TP = testosterone propionate, EsO = estrogen ointment, Th = theelin, PB = Progynon B, G = gonadin, Gg = gonadogen.

^b Numbers in parentheses are interpolations from figure 3.

^c Average of two animals.

^d Melanophores counted in the gubernaculum rather than in the parietal tunica vaginalis.

which is on the borderline of significance. If, however, the values of days 83 and 100 are excluded from this comparison because of the radical changes in the anatomical configuration during days 74 to 77, the P-value for days 24 to 40 inclusive becomes 0.0012. This is a highly significant figure and indicates that, within that period, androgens increase melanophore density.

Estrogen treatment. The only exception to the considerable melanophore increase induced by estrogen treatment is an 83-day-old treated animal. This individual, the 83-day androgen-treated animal, and the 83-day normal control are littermates; hence individual variation should be less than expected in non-littermates. In the estrogen-treated, as in the androgen-treated animals, the deletion from the analysis of animals older than 74 days changes the P-value in the direction of significance (from 0.1020 for days 17 to 83 to 0.0248 for days 17 to 43). It is possible that the mechanism bringing about the apparent decrease in normal animals between days 52 and 83 is accelerated by the administration of androgens and estrogens. Whether this is a total pigment cell reduction throughout the tunica or merely a local decrease due to stretching and redistribution is not known.

Gonadotropin treatment. The data immediately suggest an inhibitory action of gonadotropins when they are administered prior to melanophore differentiation since three animals treated before day 15 show no pigment and one treated from day 15 to day 29 has only half as many melanophores as a normal control. However, one out of three 15-day normals was previously noted to have no pigment; therefore its absence in two 15-day Gonadin treated animals cannot be attributed to the hormone. The 17- and 21-day treated individuals, as well as two similarly treated females, are littermates — and none of the four possess melanophores. There is thus a strong indication that the factor involved is genetic rather than hormonal. No other littermate is extant to furnish further information on this point. Conclusions cannot be drawn regarding the effect of the administration of gonado-

tropin on melanophore number in young opossums. Since the ratio of the 55-day animals so closely approaches unity, it is most probable that the 30-day ratio is attributable to individual variation. Moore and Morgan ('43), using the prostate as end-organ, found that the testes respond to gonadotropic treatment by precocious production of hormone by day 70 and possibly as early as day 63. From their results a gonadotropic effect would not be expected at day 55.

Castration. Melanophore density increases after castration. This result was not anticipated. Since androgens have been shown to increase melanophore density, castration would be expected to have no effect on melanophores if the testis is not producing hormone and to decrease their number if the testis is producing hormone.

The difficulty of determining a castration effect is due both to the impossibility of obtaining a total melanophore count and to the lack of meaning of density comparisons in the gubernaculum proper or in the region of its attachment to the parietal tunica vaginalis once the testis has reached the scrotum. Whereas only a short cone of cells extends from the epididymis to the saccus wall in 107- and 122-day normals, a longer and broader ligament is found in similar-aged castrates. That a mechanical stretching by the testis of the saccus vaginalis results in a shortening of the gubernaculum by a reflection of its substance into the saccus wall is certain from the studies of 107- and 122-day animals which had been unilaterally castrate for 35 and 50 days, respectively. The scrotal sac on the side from which the testis had been removed was extremely shrunken in each instance, and the gubernaculum and saccus vaginalis more closely resembled the condition in complete castrates than that in the intact side.

Melanophore type. It is impossible to follow the differentiation of an individual melanophore, but observations indicate that the following sequence occurs. Pale, yellow-brown granules appear in the vicinity of the nucleus in mesenchymal cells which are otherwise indistinguishable from fibroblasts. The granules soon pervade the cytoplasm and bring to view

the protoplasmic extensions, which may or may not have been previously present. A darkening of the melanin proceeds until the cell becomes intensely black.

Death of the cell is first evidenced by migration of granules from the processes and their ensuing paling. Occasionally cellular disintegration precedes melanin removal, and free granules are left in the intercellular spaces.

The index of melanophore type, based on the average color of the melanin granules and the extent of the cell processes, is graphed for ten normals in figure 3. Since new pigment cells are constantly being added to those already formed, an index of 9, indicating "maturity", is never reached. By day 44 the characteristic melanophore population is attained.

Although the administration of androgens and estrogens increases melanophore number, the effect of these hormones on differentiation and ultimate cell type is less evident. The data from three normal and seven treated individuals of close enough age to warrant direct comparison are presented in table 3. In addition to index number the total percentages of extreme black and of long melanophores are given. The 30-, 31-, and 32-day-old animals display considerable individual variation in index and degree of color. However, length of processes is consistently greater with androgenic and estrogenic treatment. On the other hand, all the melanophores of the treated 40- and 43-day animals were intensely black, but most of them possessed short or medium extensions. The low index number for the estrogen-treated individual seems to be due to the rapid induction and blackening of melanophores without a development of processes. This may indicate melanin immobilization; i.e., cytoplasmic rearrangement may be impossible when the cell is packed with pigment granules.

Testis descent. It will be recalled that in the normal animal the testes are inguinal by day 31, close to the scrotum by day 52, and entering the latter by day 74. However, they were found within the abdominal cavity in three androgen-treated animals of ages 35, 55, and 100 days. All of these received treatment from day 3 to days 34, 54, and 99, respec-

tively. Descent appeared normal in a 32-day (treatment days 16 to 31) and a 40-day (treatment days 30 to 39) animal. On the other hand, one individual which received testosterone propionate from day 10 to day 31 and was killed on day 39 was much farther advanced in respect to testis descent than were similarly aged normals. Ordinarily the saccus vaginalis enters the scrotal swelling between days 52 and 74, but in this animal the saccus was already within the greatly enlarged scrotum.

TABLE 3
Average melanophore type in the parietal tunica vaginalis.

AUTOPSY AGE IN DAYS	INDEX NUMBER			PERCENTAGE OF BLACK MELANOPHORES			PERCENTAGE OF LONG MELANOPHORES		
	Normal	Treated		Normal	Treated		Normal	Treated	
		Andro.	Estro.		Andro.	Estro.		Andro.	Estro.
30	..	5.8	5.9	..	35	47	..	52	60
31	4.4	7.0	5.5	48	76	47	32	63	51
32	5.8	5.8	..	94	30	..	32	70	
40	6.3	5.3	..	69	100	..	41	13	
43	..	..	3.8	..	..	100	..	..	5

That testis descent can be prevented by large dosages of androgens has been noted by Burns ('39 b, d). He suggests that though normal gubernacular attachments exist, extreme uterine development blocks descent. The administration of testosterone propionate from day 2 to day 32 in dosage so low that heterotypic organs were not affected resulted in testicular descent (Burns, '42 a, b). Since the exact location of the gonad is not reported, and since it is normally inguinal by day 31, whether or not descent was precociously induced is not known.

An obvious androgen-induced hypertrophy of the scrotum was seen in the present material only in the animal showing precociously descended testes. Though Burns ('39 a, c, e) has found no stimulatory effect of androgens or estrogens upon the scrotum of the Virginia opossum, Bolliger and Carrodus

('40) report a hastening of scrotal development in pouch young of the Australian opossum treated with testosterone propionate.

The available estrogen-treated animals do not include any treated from an early age to a time past the onset of testis descent. No obvious modifications were noted in the existing series. (See table 2 for duration of treatment and autopsy age.) The injection of estrogens has been shown to cause relaxation and wrinkling of the scrotum of *Trichosurus vulpecula* (Bolliger and Carrodus, '39).

Effect of treatments on the female

Number of melanophores. The estimated total number of melanophores in the round-bodies of fourteen normal female opossums is graphed in figure 4. From their first appearance in this structure at 13 to 16 days they increase in number gradually until day 31 and then rapidly until day 67. That individual variation is extreme is clearly evident; e.g., the sharp rise between days 31 and 32 is probably not attributable to an actual 24-hour increase. The number of animals employed is too small to permit definite conclusions regarding the exact age at which maximum pigmentation is reached, but the data indicate that it is between days 70 and 100.

It was necessary to derive melanophore number from the normal graph in instances where animals of the same age as the treated ones were not available for study. This point, as well as the great individual variation, must be kept in mind when making direct single case comparisons in table 4.

Androgen treatment. Eight out of ten animals receiving androgenic hormones possessed more melanophores than normals of comparable age. Since in the 16-day animal melanophore development has just begun, hormonal treatment from day 3 to day 15 could not be expected to affect melanophore number. Although too much weight must not be placed on any pair of figures, the remaining data, which give a P-value of 0.033, definitely suggest an acceleration of pigment cell differentiation when androgens are injected. The greatest hor-



Fig. 4 The total number of melanophores and relative weights of the round-bodies of normal female opossums.

TABLE 4

Total number of melanophores and relative weights^a of round-bodies.

AUTOPSY AGE IN DAYS	TREATMENT ^b	NUMBER OF MELANOPHORES			WEIGHT OF ROUND-BODY		
		Normal	Treated	Ratio T/N	Normal	Treated	Ratio T/N
Androgen-treated							
16	3-15 TO	116	92	0.79	0.27	0.23	0.89
22	10-21 TP inj.	(130) ^o	3780	29.08	(0.46)	0.71	1.54
24	3-24 TO	165	448	2.72	0.48	0.41	0.85
30	3-15 TO; 16-29 TP inj.	(240)	7608	31.70	(0.61)	1.30	2.13
32	16-31 TP inj.	8984	8220	0.91	1.07	1.37	1.28
39	10-31 TP inj.	(10200)	18420	1.81	(1.80)	2.01	1.12
45	3-15 TO; 16-44 TP inj.	(10600)	26805	2.53	(2.15)	2.45	1.14
62	10-31 TP inj.	(22200)	27186	1.22	(2.10)	2.41	1.14
95	3-44 TP inj.	(32000)	43704	1.37	(5.40)	3.14	0.58
	3-93 TP inj.		52695	1.64		6.54	1.21
Estrogen-treated							
17	5-16 EsO	36	828	23.00	0.39	1.11	2.84
21	5-20 EsO	(110)	1588	14.44	(0.44)	1.46	3.32
22	6-21 EsO	(130)	595	4.58	(0.46)	4.14	9.00
24	6-23 EsO	165	4202	25.47	0.48	2.65	5.52
30	6-29 EsO	(240)	23656	98.57	(0.61)	4.61	7.56
39	6-38 EsO	(10200)	32192	3.16	(1.80)	6.87	3.82
43	6-42 EsO	(11100)	47225	4.25	(2.20)	7.62	3.46
79	6-45 EsO	(26300)	28919	1.10	(2.70)	6.55	2.43
Gonadotropin-treated							
15	8-14 G inj.	0	0		(0.24)	0.21	0.88
17	10-16 Gg inj.	36	0		0.39	0.38	0.97
21	10-20 Gg inj.	(110)	0		(0.44)	0.53	1.20
30	15-29 Gg inj.	(240)	2616	10.90	(0.61)	0.61	1.00
38	15-37 Gg inj.	(10000)	10206	1.02	(1.70)	1.12	0.66
80	54-79 G inj.	(26000)	15310	0.59	(2.80)	2.85	1.02
Castration							
53	castrate day 20	(12800)	9006	0.70	(1.90)	1.90	1.00
83	castrate day 40	25413	7715	0.30	2.33	1.30	0.56
103	castrate day 26	36465	13392	0.37	7.57	2.08	0.27
110	castrate day 20	22591	11960 ^o	0.53	8.99	3.07 ^o	0.34
adult	castrate for 74 days	30562 ^a	8797	0.29	53.38 ^a	41.19	0.77
	castrate for 60 days		10583	0.34		30.26	0.57

^a Weights are expressed in arbitrary units for comparative purposes only. Actually they are the weight in grams of the traced sections.

^b Abbreviations are as follows: TO = testosterone ointment, TP = testosterone propionate, EsO = estrogen ointment, Gg = gonadogen, G = gonadin.

^c Numbers in parentheses are interpolations from figure 4.

^d Average of two animals.

^e Average of three animals.

monal effect is exerted in the earlier period of pigment cell production.

Estrogen treatment. More clear-cut evidence suggests that the administration of estrogens increases total melanophore number. In every case which was studied, the treated animal possessed many more pigment cells than did a similarly aged normal. This is reflected by the P-value of 0.0032 obtained for the eight comparisons. As noted for the androgenic treatment, the greatest effect is produced during the period when normally pigmentation is slowly increasing.

Gonadotropin treatment. The 17- and 21-day-old treated females are littermates of the previously described Gonadogen-treated males. Complete lack of pigmentation is attributed to the genetic constitution of this litter rather than to its treatment with hormones.

The melanophore ratios of treated to normal animals at 30 days are extremely high. This is due in part to the very low number of pigment cells which were present in the only normal animal of a similar age that was available for study. Figure 4 shows the extreme variation between a normal 31-day and a 32-day animal. Therefore it is felt that a ratio of 10.9 here does not indicate hormonal stimulation of pigment formation whereas ratios of 31.7 and 98.6 in 30-day animals after androgen and estrogen treatment, respectively, are significant.

Results from the 38- and 80-day treated individuals suggest that the ovaries at these ages are not responsive to treatment with gonadotropins. Moore and Morgan ('43) obtained evidence of induced ovarian secretion only subsequent to day 100.

Castration. The mechanics of ovariectomy in pouch young so distort normal relationships that often the round-body becomes embedded in the ventral body wall instead of lying free in the abdominal cavity. Though its altered position conceivably could retard or modify its development, the round-body appears morphologically and histologically normal. Castration in the adult necessitates no manipulation of this structure.

Every castrate animal studied possessed significantly fewer melanophores than normals of the same age ($P = 0.0010$). If the 53-day comparison is omitted, since it includes an interpolation for the normal value, the difference between castrates and normals is even more significant ($P = 0.0005$). Melanin production continues after day 20 in the absence of the ovaries, but the number of pigment cells does not go beyond that expected at about day 55. Likewise castration of the adult causes a decline in melanophore number to approximately the 55-day level. This argues for a purely genetic determination of melanin production at least to day 55, which is supplemented by a hormonal stimulus coming in soon after. If the assumption that the ovary has assumed its endocrine function at 55 days is correct, it would be expected that gonadotropic treatment at this time would accelerate pigment cell differentiation; yet this was seen not to be the case in an individual receiving Gonadin from days 54 to 79 and autopsied at day 80. An alternative explanation is that the altered location of the round-body resulting from the operation is responsible for the diminished number of melanophores. However, this does not account for the reduction noted in adult castrates.

Size of the round-body. Relative sizes of round-bodies were estimated as previously described by tracing and then weighing the cut sections. Figure 4 graphs the weight increase in a series of normal animals. Individual variation is considerably less in round-body size than in melanophore number. The rate of increase, which is more or less gradual up to day 83, is accelerated thereafter. Contrary to melanophore number, which remains substantially constant after day 67, there is a five-fold growth of the round-body from day 110 to the adult. The consequent dispersal of melanophores accounts for the apparent paling and obscurity of the adult round-body.

Androgen treatment. There seems to be no effect of androgens on round-body size. The geometric mean of the ratios of treated to normal is 1.15; $P = 0.26$.

Estrogen treatment. The great growth of the round-body under the influence of estrogens ($P < 0.0002$) is due to the proliferation of cells typical of this structure.

Gonadotropin treatment. In the ages studied gonadotropins have no effect on round-body size. The geometric mean of the ratios is 0.94; $P = 0.48$.

Castration. Every castrate studied, with the exception of the 53-day animal, possessed smaller round-bodies than normals of the same age. The P -value for all six comparisons is 0.0251; significance becomes even greater ($P = 0.0163$) when the 53-day individual is omitted from the comparison.

The round-bodies of ovariectomized pouch young never attain normal dimensions although they continue to grow to the approximate size expected in 55-day individuals; those of adults show definite involution 60 days after castration. These data can be explained by assuming direct genetic determination up to about day 55 with additional hormonal stimulation coming in soon after. However, it is possible that in early castrated animals the position in the body wall of the round-body may partially account for its growth inhibition.

Number of melanophores per round-body unit weight. Since the results of hormonal effect on total melanophore number and round-body size are so similar, it became necessary to determine whether the former might be a function of the latter — i.e., the increase in melanophore number conceivably could be dependent upon the increase in round-body size rather than upon the hormone directly. If so, the number of melanophores per round-body unit weight should remain relatively constant.

Analysis shows that in normal animals the density of melanophores increases twenty-five-fold from their first appearance until day 83. Subsequent round-body growth without accompanying increase in pigment cell differentiation pulls down the ratio until in the adult melanophore density approaches that in animals 30 days of age or younger.

With androgen treatment the average melanophore density is five times that of normals; with estrogen treatment it is

four times as great. Since there is no increase in round-body size with the former treatment and a significant increase with the latter while total melanophore number rises with each, it may be concluded that we are dealing with two separate hormonal effects.

That round-body size may limit the total number of melanophores must not be overlooked. Though no estrogen-treated animal approached the density observed in an 83-day normal, this figure was exceeded in two androgen-treated individuals. This suggests the possibility that melanoblast response to androgens may be restricted by size limitations of the containing structure.

SCROTAL PIGMENTATION OF THE GROUND SQUIRREL

The seasonal coloration of the scrotal skin of the ground squirrel is due to the recurrent presence of melanophores at the dermal-epidermal junction. These large, dendritic cells, packed with melanin granules, closely resemble those seen in the gubernacular apparatus of the opossum with the exception that the latter seem to be oriented in response to the stresses and strains imposed on their containing tissue while the former are confined to the interface between dermis and epidermis. The processes of the pigment cells stretch out along the basement membrane or actually penetrate the epidermis (fig. 17). Melanin granules, probably transmitted to the epidermal cells by way of these processes, lie primarily above their nuclei. As cornification proceeds the granules tend to disappear, although many remain in the stratum disjunctum and are finally lost by desquamation.

The morphological differences between scrotal pigmentation and that of the general integument are quantitative rather than qualitative.

Seasonal variation of scrotal color

Adult field animals. In the Chicago region male ground squirrels emerge from hibernation during the first 10 days in April. Some are capable of mating immediately while others

do not attain the ability to copulate until a few weeks later (Moore et al., '34; Wells, '35). The scrota at the end of hibernation are dark, become even blacker during May, and then pale until no pigmentation remains when the animals return to hibernation in October.

In figure 5, based on forty-nine animals autopsied 1 to 3 days after capture, are shown the weights of testes, seminal vesicles, and prostates, and the degree of scrotal pigmentation classified on an arbitrary scale from "a" to "f". Though the testes are maximal at or shortly after emergence and rapidly regress thereafter, the seminal vesicles and prostates attain their greatest weights in late April or early May. Complete sexual quiescence is indicated from early July until hibernation. These data do not differ significantly from those obtained by others working in the Chicago region (Wells, et al.).

The similarity in the behavior of scrotal pigmentation to that of the accessory reproductive organs strongly suggests that it also is under gonadal control. That it is an even more sensitive indicator of testis secretion than the seminal vesicles is evidenced by the fact that, when the animals first appear in spring, pigmentation is relatively further advanced than are the seminal vesicles. The peak of scrotal color, however, is reached 7 days later than that of seminal vesicle weight, probably because of the greater length of time required for melanin production.

Extreme pigmentation is maintained from May 5 to June 3. There are two possible explanations for the presence of maximal scrotal color past the onset of seminal vesicle regression and its retention as late as September. (1) If, as is assumed above, the melanophores have a lower threshold of response to gonadal hormones than the seminal vesicles, it is possible that pigmentation is maintained by a very low level of testis secretion. Although testis weight has greatly decreased, its hormonal activity has not necessarily entirely ceased. (2) The elimination of existing melanin may require considerably more time than ordinary tissue involution.

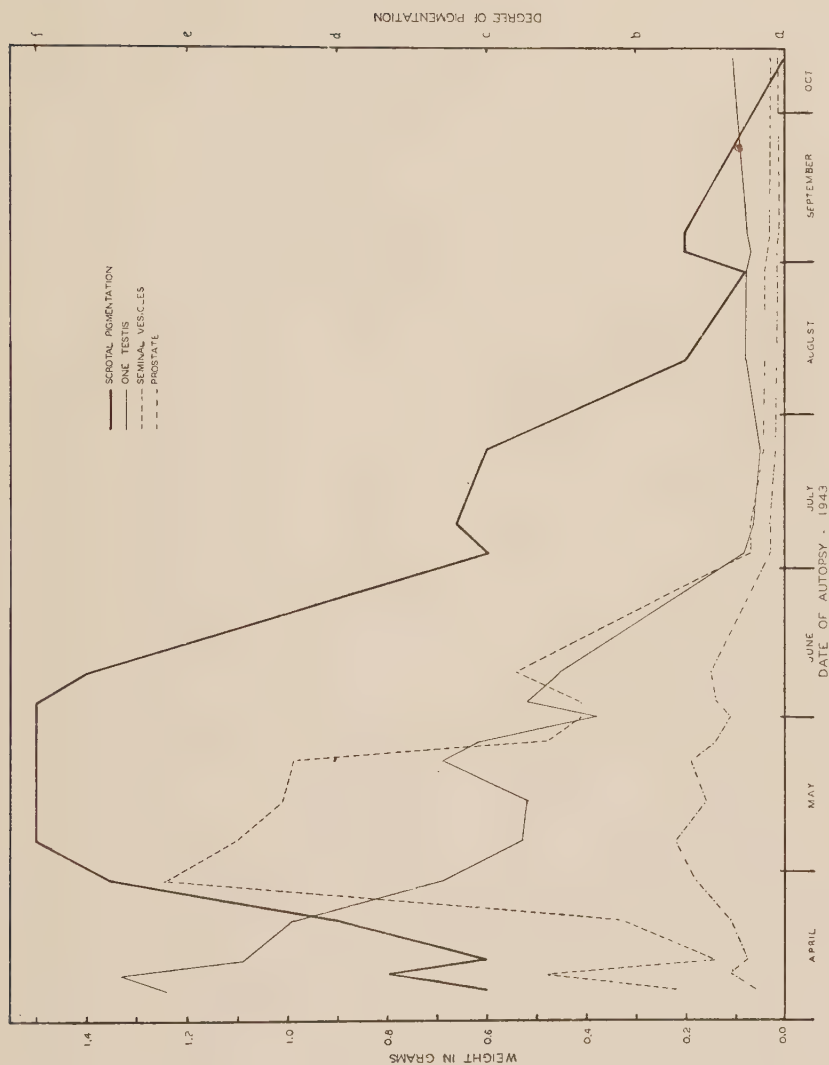


Fig. 5 Scrotal pigmentation and testis, seminal vesicle, and prostate weights of adult field ground squirrels.

Adult laboratory animals. That non-hibernating male ground squirrels confined to the laboratory come into apparent reproductive activity earlier than field animals has been noted by Wells ('35). Repeated observations of pigmentation in a total of seventy-two laboratory animals (6 to 17 months after capture) disclosed not only that the degree of scrotal darkening in the middle of February was comparable to that seen in April in field animals but also that reduction of pigmentation at the end of the breeding season lagged 20 to 50 days. Scrotal color of "c" or darker persisted for 7 months in laboratory animals but only for $3\frac{1}{2}$ months in field animals. Constant breeding condition can be maintained by inducing hibernation during the mating season (Wells and Zalesky, '40). An experimental group of hibernating animals kept at 4°C. by Dr. G. F. Simmons was observed from time to time; the scrota continued to be black.

The laboratory data thus furnish further evidence that the degree of scrotal pigmentation is a function of testicular activity.

Response to hormonal treatment and to castration

Adult castration. Four animals served in a preliminary experiment to determine the effect of castration upon scrotal pigmentation. Two were castrated 6 days after their capture on April 30, when the seminal vesicles are maximally developed and scrotal color is approaching its extreme. All showed pigmentation of 1.4 on the "a-f" scale at the onset of the experiment. One pair (control and castrate) was killed 27 days later and the other 43 days after operation.

The reduction of pigmentation was obvious 18 days post operation; the colored area was reduced in size and grey rather than black. Pigmentation decreased by more than half in 27 days and by almost four-fifths in 43 days. The fact that detectable scrotal color remains 43 days after castration makes it not unlikely that the retention into September of small amounts of pigment as previously noted in field animals is due to the sluggish elimination of melanin.

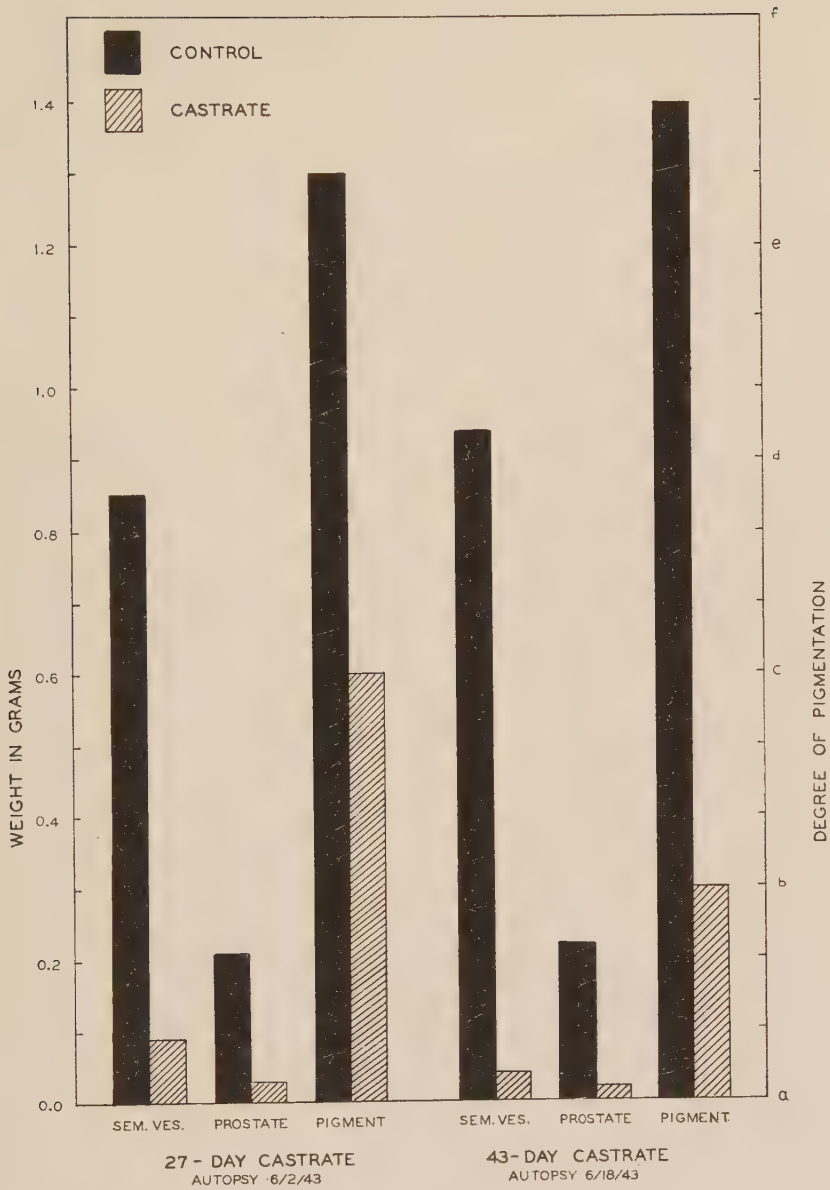


Fig. 6 The effect of castration of reproductively active male ground squirrels on scrotal pigmentation, seminal vesicle weight, and prostate weight.

Treatment of immature animals. An experimental group of twenty-seven immature male ground squirrels provide additional proof of the testicular control of scrotal pigmentation. The data obtained from fourteen of these are presented in table 5. Animals were collected July 29 and August 8, and experimental treatment began with castration on August 21 and subcutaneous hormone injection the following day. Since the extreme dates of littering in the Chicago region are May 23 to June 10, with the mode falling on June 1 (Moore et al., '34; Simmons, personal communication), these animals can

TABLE 5
Effect of treatments on immature ground squirrels.

NO. OF ANI- MALS	TREATMENT	BODY WEIGHT IN GRAMS		WEIGHT OF GLANDS IN MILLIGRAMS			SCROTAL COLOR (SCALE = 0.0-1.5)
		Initial	Autopsy	Testes	Sem. ves.	Prostate	
4	Control	121	143	74.7	6.2	3.0	0.0
2	Castrate	110	121	(55.2) ^a	5.8	2.5	0.0
2	130 r.u. gon.	125	175	370.1	262.7	91.5	0.9
2	Castrate						
	130 r.u. gon.	122	158	(62.0) ^a	6.4	3.1	0.0
2	1300 gamma						
	TP	114	151	193.0	508.7	125.8	1.3
2	Castrate						
	1300 gamma						
	TP	115	146	(59.4) ^a	562.0	133.4	1.3

^a Testes weight at time of castration.

be estimated to be $2\frac{1}{2}$ to 3 months old at the onset of treatment; the experiment was terminated 14 days later.

Since castration alone of such immature animals has no effect on the accessory reproductive glands, it can be concluded that testis secretion either has not yet begun or has already ceased. However, the capacity of the testis to produce hormone at this time is made clear by the great development of the seminal vesicles and prostate under Gonadin treatment. Spermatogenic activity is precociously induced by the administration of both Gonadin and testosterone propionate.

The short duration of treatment did not permit spermatid formation, but it has been demonstrated that spermatozoa can be produced by the injection of gonadotropins and androgens as early as August in immature ground squirrels (Wells, '35; Wells and Moore, '36).

Scrotal darkening was first observed 4 to 6 days after the onset of treatment either with Gonadin in the intact animal or with testosterone propionate in the castrate and non-castrate. Throughout the experiment there is a parallel response of accessory reproductive organs and scrotal melanophores.

Juvenile pigmentation

Although the integument of adult ground squirrels is grey-pink, that of immature males and females varies from dark grey in early summer to the adult color in autumn. The scrotal region is involved as well as the entire body, and there is no sharp line of demarcation between black scrotum and "colorless" integument as is found in reproductively active adults. The melanophores responsible for the pigmentation do not differ morphologically from those which color the adult scrotum. They lie at the dermal-epidermal junction, send their processes along the basement membrane and into the epidermis, and appear to deposit melanin granules in the epidermal cells. Sections of skin from the back, belly, and scrotal region are indistinguishable.

The pigmentation of young ground squirrels has not escaped attention. Wade ('27) mentions killing one animal at an early age to study microscopically the color he observed in animals 8 days of age, but he does not state his finding. Johnson ('31) noted that color first appeared at 3 days and that it deepened at 4 to 6 days.

Figure 7 is based on data from fifteen immature male ground squirrels autopsied 1 to 3 days after capture. Pigmentation of the scrotal region, classified on the previously described "j0-j8" scale, is seen to be highest when the animals first venture from the nest and then gradually to regress until none is left in October. Since in a single 72-day-old litter

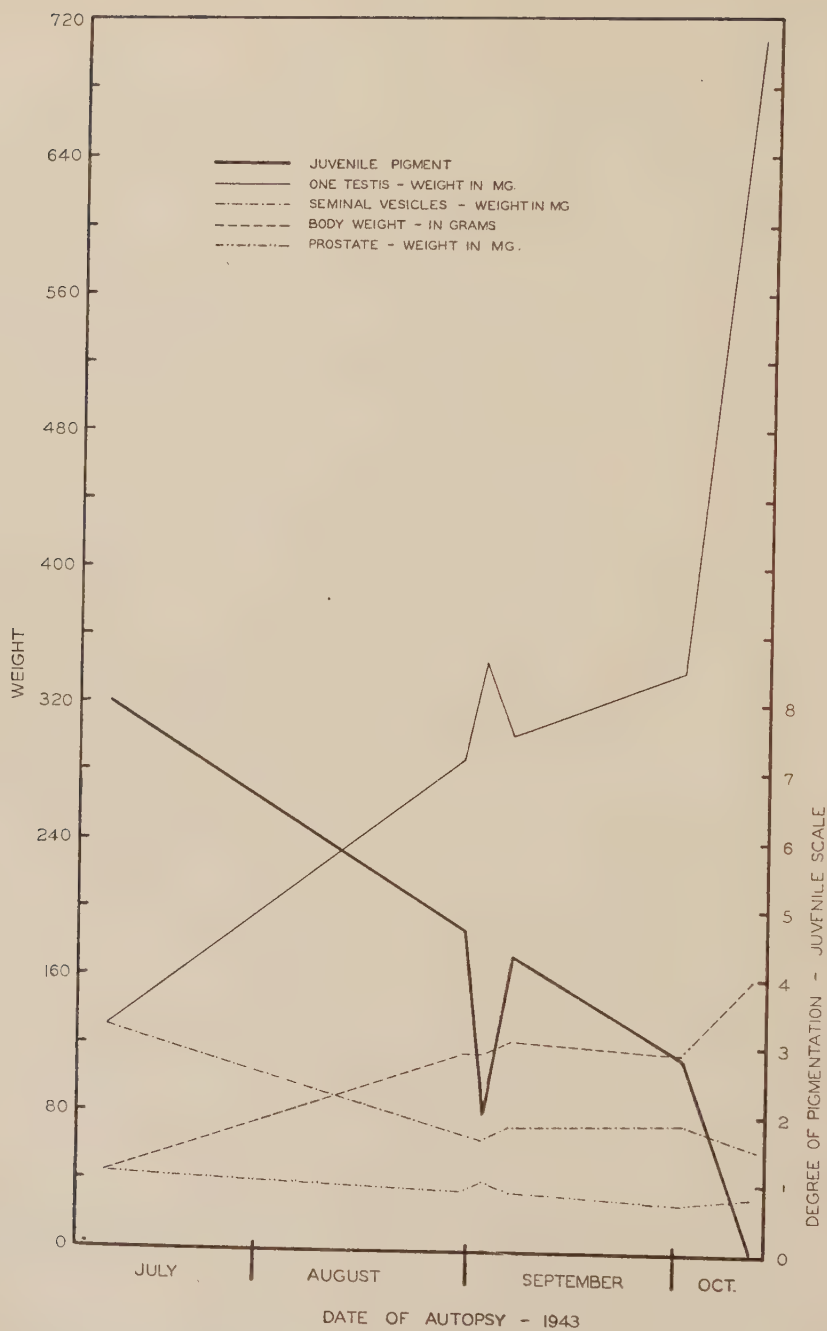


Fig. 7 Juvenile pigmentation in relation to body weight and testis and accessory weights in immature ground squirrels.

described by Johnson skin color varied from dark to unpigmented, age cannot be the determining factor. There is, however, a strong negative correlation ($- .80$, probability less than $.01$) between body weight and skin color. It should be noted that, despite the rapid increase in testicular weight from early July until the first hibernation, the seminal vesicles and prostate become lighter.

The injection of estrogens and gonadotropins into young females before or during pigment regression does not alter the normal sequence of events; paling begins in the posterior mid-ventral region and gradually extends anteriorly and dorsally until the adult color is attained (Simmons, unpublished data). Nor does the administration of androgens or gonadotropins retard pigment regression in the male except in the scrotal region; early castration has no effect on male juvenile pigmentation. Therefore it must be concluded that the general integumentary melanophores, although morphologically indistinguishable from those which color the scrotum of the reproductively active adult, are physiologically different.

DISCUSSION

In 1912 Weidenreich advanced the hypothesis that all vertebrate melanophores are homologous and are of neural crest origin. Although it has now been conclusively demonstrated that amphibian and avian pigment cells are derived from the neural crest (cf. reviews by DuShane, '43, '44), the problem of the embryonic origin of mammalian pigment cells is still not solved. The majority of investigators are of the opinion that in mammals melanophores develop either from modified connective tissue cells or from specialized mesodermal cells, and that the epidermal cells produce their own pigment (Biederman, '28; Vilter, '35). At present, "Comparative grounds alone make the assumption likely that vertebrate pigment cells have a common embryological origin" (DuShane, '44, p. 113). To the extent that the present study indicates points of similarity between the melanophores of birds and those of mammals, it furnishes additional evidence in

support of the hypothesis that all vertebrate melanophores are homologous.

Examination of the whole integument and especially the scrotal skin of the ground squirrel reveals that the melanin eventually lost by desquamation is not produced by the epidermal cells, as has been suggested, but merely transferred to them via the long processes of melanophores. The cells themselves are stretched out along the epithelial basement membrane, but their extensions penetrate the epidermis and make their way among the epidermal cells. A similar relationship of dermal melanophores and epidermal melanin granule inclusions was noted in those few regions where epidermal color (other than that in hair follicles) appears in the opossum — the sole of the hind foot and dark band encircling the tail. Occasionally in the black spots of the ear, where dermal pigmentation is so intense that melanophores appear to outnumber connective tissue cells, isolated melanin granules occur in one or two epidermal cells. One receives the impression that the mechanism which here normally prevents the transfer of melanin to the epidermis now and then breaks down under the impact of the extreme dermal pigmentation. Cursory study of hair follicles in both animals likewise suggests a transference of melanin granules into the developing hair by melanophores which have come to lie within the follicle. These observations coincide with the known method of feather pigmentation and lend support to the recent suggestion of Willier ('42 b) that melanophores supply pigment to the epidermal cells of the hair. The first description of such a process in man was given by Riehl (1884).

The distribution of pigment cells has undergone progressive restriction in vertebrate phylogeny. The melanophores found in association with blood vessels, peritoneum, mesenteries, viscera, and central nervous system of the lower classes are all but lacking in birds and mammals (Weidenreich, '12; DuShane, '43). Integumentary pigmentation as well has diminished in the deep layers of the dermis although melanophores persist in the dermal-epidermal junction and hair follicles of

mammals and developing feather germs of birds. This evolutionary sequence suggests that protective feathers and hair supplant deep-lying pigmentation.

The remarkable retention of gonad pigmentation in some chicken breeds (Minoura, '21; Willier and Yuh, '29) and in the European starling (Bissonnette, '30) parallels the situation in the opossum and ground squirrel. Although the location of pigment cells in these three cases differ — within the gonad proper, in the parietal tunica vaginalis, and in the scrotal skin, respectively — the end result is the same; i.e., the germ cells are inclosed within a pigmented sheath. Is this merely coincidental or is some evolutionary advantage actually involved? The fact that three unrelated animals display analogous pigment retentions in spite of the afore mentioned evolutionary regression is suggestive. If mammalian melanophores originate in the neural crest, and if melanoblasts migrate into all regions of the developing organism, why should the parietal tunica vaginalis of marsupials and the scrotal skin of rodents furnish the necessary environment for the elaboration of pigment, as does the bird gonad, unless these animals are in this way somehow more favorably endowed? It is true that the entire epidermis of the ground squirrel is supplied with melanophores, but nowhere in such abundance as in the scrotal skin. *Citellus* is not peculiar among rodents in this respect; in new-born black and brown mice the first color appears in the scrotal region and remains obviously darker here at least until the growing hair masks the skin. Reed ('38) states that in hairless mice (and probably guinea pigs as well) pigment occurs only in the eyes, ears, and scrota.

The nature of any adaptive value provided the gonad by pigmentation is obscure. The prevention of light penetration seems unlikely since the opossum is nocturnal and the period of greatest spermatogenic activity in the ground squirrel occurs while the animal is in hibernation. Moreover, Seelig ('13) showed that the deleterious effects of light are no greater on grafts of white guinea pig skin than on black. Loeb (1897) suggested that pigmented skin is less sensitive than non-pig-

mented since it is less subject to desquamation. Testicular protection might be provided the ground squirrel in this manner. With the present material it was repeatedly observed that near the end of the period of color regression flakes of black cornified tissue were being lost instead of the usual macroscopically imperceptible cornified cells.

No instance of the absence of scrotal color in reproductively active ground squirrels was encountered. Such deficiency, if it occurred or could be induced experimentally, might give some indication of pigment function in this animal. Microscopic study of the four opossums which lacked pigment in one or both vaginal sacs revealed no other abnormality of parietal tunica vaginalis, gubernaculum, epididymis, or seminiferous tubules. However, this does not exclude the possibility of a physiological deviation from normal. No function can be attributed to the female round-body; it is merely a vestigial homologue made conspicuous by its deep color.

Strikingly similar are the cyclical nature and hormonal control of English sparrow bill color and ground squirrel scrotal pigmentation. The bill is horn-colored in winter and becomes black during the breeding season; castration in summer causes a complete paling in 6 weeks, and the color can be quantitatively restored by injecting androgens (Keck, '34). The administration of gonadotropic substances during the sexually quiescent period changes bill color from light to black in 3 weeks (Witschi and Keck, '35). These findings closely coincide with the present data dealing with ground squirrel scrotal pigmentation. Seasonal variation in color parallels the state of reproductive activity; castration at the height of the breeding season causes a regression of pigmentation; androgen treatment induces coloration in sexually quiescent or castrate ground squirrels; and gonadotropins administered to reproductively inactive animals leads to the production of black scrota. Histological similarities also are present. Witschi and Woods ('36) describe melanophores attached to the epidermal basement membrane in the sparrow bill which furnish the growing cells with melanin granules. Thus the

cornified cells, which never produce pigment, become packed with melanin provided by the hormonally stimulated pigment cells. It appears that the mechanism of pigmentation is the same in the ground squirrel scrotal skin. The androgen sensitivity of the sparrow bill has made this secondary sex character a useful male hormone indicator (Witschi, '36) just as the degree of scrotal pigmentation has served as an indicator of the reproductive state in the ground squirrel.

Gonadal control of other integumentary melanophores has been demonstrated also. The influence of androgens and estrogens upon plumage coloration in sexually dimorphic birds has been intensively studied. (For an introduction to the extensive literature dealing with this subject see Domm, '39; Danforth, '42; Willier, '42 a.) Red and black bird melanophores grown in tissue culture likewise respond to sex hormones by increased or decreased differentiation (H. L. Hamilton, '40, '41). Mammalian pigmentary changes are less widespread and can be attributed definitely to a stimulation by sex hormones in only a few instances. Kupperman ('44) studied the small pigmented area in the dorsolateral subcostal region of the male golden hamster and found that the color, which decreases after castration, can be maintained by the administration of androgens. Female hormones have no effect in the male except to indirectly decrease pigmentation through inhibition of the pituitary gland. Corresponding skin in the female, which normally lacks pigment or is very light, can be induced to take on male characteristics if androgen treatment follows ovariectomy. J. B. Hamilton ('36) has differentiated a reddish-yellow region in the ventro-caudal portion of the albino rat scrotal skin. He states that color of this "sexual skin" fades and is sloughed off after castration or hypophysectomy. No attempt was made to determine the origin, histologic location, or character of the pigment. Bolliger and Carrodus ('40), working with *Trichosurus vulpecula*, found that androgen treatment of males resulted in the secretion of pigment around the tip of the scrotum and estrogen treatment of females caused a similar pigmentary increase in the marsupium.

Areolar and nipple darkening has frequently been noted in lower mammals and in women during pregnancy. Bloch and Schraffl ('32) were able to induce a similar mammillary darkening in normal and castrate male guinea pigs by injecting or feeding estrogens. The effect which they attribute to a direct response of melanophores (actually melanoblasts in the dual terminology of Bloch, '17) to female sex hormone is assigned instead to indirect action through the posterior pituitary gland by Vilter ('33). J. B. Hamilton ('37, '39) has offered some clinical evidence of gonadal control of skin pigmentation in man. Androgen treatment resulted in the disappearance of the dark regions under the eyes characteristic of hypogonadal men. The hands, head, and neck of five women receiving estrogens became darker, and the previously attained tan of one took on a deeper hue. Androgen treatment in men likewise was shown to increase the depth of sun-tan acquired prior to the onset of treatment (J. B. Hamilton and Hubert, '38). These authors suggest that sex hormones "develop" the rather colorless material laid down in the skin after exposure to light.

Analysis of color variation in the general body skin of adult male ground squirrels has not yet been undertaken. However, a slight paling of dorsal and belly regions from early spring to midsummer was evident. Whether or not this phenomenon will prove to be linked with gonad activity can only be surmised. Similarly, the underlying factors governing juvenile pigmentation require further investigation. These may be entirely genetic or may be hormonally augmented. Pure genetic control implies that the formation and regression of melanophores are not controlled by the secretory activity of the endocrine glands; hormonal control presupposes the proper genetic background accompanied by an effect directly attributable to an endocrine secretion. The inability of juvenile pigment cells of the general integument to respond to sex hormones suggests that they are fundamentally different from the rapidly activated scrotal melanophores. They may be an entirely unrelated generation of short-lived cells which

differentiate early and soon disappear. On the other hand, it may be that after the first surge of melanin production the capacity to respond is lost by all but the scrotal melanophores. The latter hypothesis requires the presence of a circulating hormone very soon after birth — a contention which is supported by the decrease in the weights of the accessory reproductive organs that begins at the time the animal first leaves the nest and that continues until hibernation. A similar reduction in the accessory reproductive organs in immature females has been observed by Simmons (unpublished data), who interprets it as an indication that the gonad, which has already assumed its endocrine function, soon ceases hormone production in response to the same factors which control retrogression in mature individuals. Although the immature gonad can be activated by gonadotropins, there is no proof that under normal conditions secretory activity is begun during the first season. It is not known whether or not scrotal color can be induced by the injection of estrogens; however, the presence of entirely comparable juvenile pigmentation in both males and females suggests a common stimulus rather than androgen activation in one and estrogen activation in the other.

As far as is known, deep-lying melanophores associated with the reproductive tract occur in no mammal other than marsupials. Although they first appear adjacent to and in the peritoneum of the ventral body wall of the opossum, they are restricted to the parietal tunica vaginalis of the adult male and the round-body of the adult female. Since melanoblasts cannot be detected prior to melanin production, their origin remains obscure. The most faintly pigmented cells differ structurally from fibroblasts only in their content of yellow-brown granules. No evidence beyond that based on comparative ground supports the contention that these melanophores and those of the skin and hair are embryonically similar. Several points of physiologic and morphologic differences may be noted. (1) Gubernacular melanophores are present 15 days after birth; integumentary pigment was observed first

in a 32-day hair follicle. (2) Melanophores of the dermo-epidermal junction, and probably the hair follicles as well, appear to transfer their pigment to epidermal cells; whether or not the deep-lying pigment cells have a similar capacity is unknown since they are not in contact with epidermis. (3) Integumentary pigmentation does not respond to sex hormone administration or to castration, whereas there is an undeniable effect of treatment upon pigmentation associated with the reproductive tract. Both androgens and estrogens increase the total number of melanophores in the female round-body, while ovariectomy results in pigment reduction. Although total melanophore number could not be ascertained in the male, it has been shown that their density in the most deeply colored portion of the parietal tunica vaginalis is increased by androgen and estrogen treatments.

Other hormonal effects on the gubernacular apparatus beside those concerned with pigmentation deserve mention. The extreme increase which is induced in the size of the round-body by the administration of estrogens and the decrease which follows castration indicate that this vestigial organ is normally maintained by the ovary. Since both round-body size and total melanophore number in early castrates do not exceed the normal 55-day level, and since ovariectomy of older animals leads to pigment cell decrease to the 55-day level, the supposition might be made that ovarian secretion occurs at this time in sufficient quantity to enhance the earlier purely genetic control. However, the possibility that these results may be due to the operation itself and not to the removal of the ovaries has not been ruled out. Such early ovarian secretion is not in accord with the findings of Moore ('43) and Moore and Morgan ('43), who were unable to detect a stimulating effect of gonadotropins in the female opossum before day 100.

The effect of hormones on the round-body suggests that the male homologue, the chorda gubernaculi, likewise may be under partial hormonal control. Unfortunately the size of the chorda cannot be readily measured. Until quantitative

studies can be made, the problem of testis descent may remain unsolved. In the opossum androgen treatments have been found to have no effect on descent, to cause abdominal retention of testes, or even to induce precocious descent.

Several extra-testicular hormonal agents which may contribute to testis descent in man have been proposed; e.g., the prenatal action of chorionic gonadotropins (Engle, '32), estrogen stimulation during pregnancy (Wislocki, '33), and activation by "the androgen of pregnancy" (Wells, '44). These hypotheses, though applicable to man, where descent normally is completed by the eighth month of uterine life (Wells, '43; Wyndham, '43), cannot explain the situation in those mammals in which descent occurs weeks, months, or years after birth. J. B. Hamilton ('38) has shown that the testis of the rhesus monkey, which is scrotal at birth but abdominal shortly thereafter and until 4 or 5 years of age, can be induced to return to the scrotum by the injection of androgens. Wyndham ('43), on the other hand, presumes that the development of the entire region concerned with testis descent is controlled by the testis under the influence of the pituitary gland.

Does testicular hormone normally play a rôle in opossum testis descent? It will be recalled that the testis has left the abdominal cavity by day 31 and slowly descends with the downward migration of the saccus vaginalis until it lies slightly above the scrotal swelling by day 52. Not until day 74 does the further penetration of the saccus permit the testis to enter the scrotum; 3 days later descent is complete. These observations suggest that shortly before day 74 there is some new impetus in addition to those responsible for the first phases of descent. Since the testes respond to gonadotropins by day 70 (Moore and Morgan, '43), it is possible that a low level of normal secretion has begun by this time. However, there is no indication of a testicular influence on the histological differentiation of Cowper's glands (Rubin, '44) and the prostate gland (Moore, '43) prior to day 100. Similarly, studies on the hypophysis suggest that there is an absence of sex hormones until day 100 (Wheeler, '43). There is

no doubt but that at least the greater part of descent continues in the absence of testicular hormone. These findings are in accord with the thesis of other individuals investigating the opossum, which has most recently been summarized by Moore ('44 a, b), that the accessory reproductive structures in the opossum owe their primary induction and development to genetic factors rather than to gonad-secreted hormones.

No satisfactory explanation of the mechanics of testis descent has been presented although numerous developmental factors have been proposed. These include (1) a softening of abdominal tissue producing a cavity through which the testis plunges (Forssner, '28), (2) abdominal pressure exerted upon the testis by the large bowel or developing viscera, (3) differential growth of body parts causing a caudal shifting of the gubernaculum, (4) upward growth of the ventral body wall to surround the testis, (5) contraction of the gubernaculum by formation of scar tissue, and (6) muscular contraction of the gubernaculum (see Eberth, '04; Wells, '43; Wyndham, '43). Emphasis has been placed on the shortening of the gubernaculum rather than on the location of the saccus vaginalis and its capacity to contain the testis. Hart ('09 a, b), however, recognized the importance of the growth of the saccus vaginalis. Although he states that the canal through which the testis passes is formed by the gubernaculum, he does not indicate how this is accomplished.

In the opossum it is the progressive penetration of the saccus vaginalis into the scrotum which carries the distal attachment of the gubernacular apparatus more deeply into the ventral body wall. The epididymis is thus drawn into the inguinal canal, and along with it the mesorchium and testis. Histological observation indicates that the enlargement of the vaginal cavity, coincident with the downward migration of the testis, is due to withdrawal of material from the gubernaculum and its incorporation into the walls of the saccus vaginalis. Lending support to this hypothesis is the fact that in the early phases of testis descent only occasional melano-

phores invest the saccus while the gubernaculum is densely pigmented; however, when descent is complete the parietal tunica vaginalis is intensely black. The primary mechanical factor of testis descent accordingly appears to be the growth and migration of the saccus vaginalis; the gubernacular apparatus merely guides the epididymis and consequently the testis. It is progressively shortened by furnishing material at its distal attachment to the enlarging parietal tunica vaginalis. The pars scrotalis, though considered to be one of the components of the gubernacular apparatus by Felix ('12) and Moszkowicz ('35), among others, does not become incorporated in the composite ligament but supplies additional substance to the wall of the migrating saccus vaginalis.

SUMMARY AND CONCLUSIONS

The opossum gubernacular apparatus

1. The gubernaculum of the male opossum is composed of the following parts: (1) the pars mesorchica, derived from the ligamentum testis; (2) the pars mesonephridica, developed in the tubar portion of the mesonephros; (3) the pars inguinalis, which results from the fusion of the inguinal fold (a ventral proliferation from the tubar portion of the mesonephros lateral and dorsal to the wolffian duct) and the inguinal crest (an evagination of subperitoneal cells of the ventral body wall lateral to the urinary bladder); (4) the pars intermuscularis, the original chorda gubernaculi, which is first observed as a proliferating mass of cells in the ventral body wall continuous anteriorly with the inguinal crest; and (5) the pars scrotalis, derived from the ligamentum scroti, which extends from the ventral integument to the chorda gubernaculi.

2. The homologous structures in the female are: (1) the ligamentum ovarii proprium (the pars mesorchica of the male); (2) the pars mesonephridica, incorporated in the tissue surrounding the uterus; (3) the ligamentum rotundum, derived from the fused inguinal crest and inguinal fold; and (4) the round-body, which arises from the anterior part of the chorda gubernaculi. A ligamentum scroti does not appear

in the female, and the caudal portion of the chorda gubernaculi disappears by the twelfth day.

3. It is likely that sex can be diagnosed prior to gonad differentiation on the basis of gubernacular development. In the "male" at birth only the chorda gubernaculi and ligamentum scroti are represented; in the "female" are found the inguinal fold, the inguinal crest, and the chorda gubernaculi with its already recognizable round-body.

4. The saccus vaginalis, which is present at birth as a very small coelomic evagination medial to the chorda gubernaculi, soon disappears in the female. In the male it penetrates the ventral body wall along the course of the chorda gubernaculi and eventually invades the ligamentum scroti lying within the scrotal swelling. Growth of the saccus vaginalis proceeds at the expense of the gubernaculum; material from the chorda gubernaculi and inguinal ligament is being withdrawn continually into the wall of the saccus vaginalis. The ligamentum scroti likewise furnishes cells to the parietal tunica vaginalis.

5. Testis descent is accomplished by the growth and penetration of the saccus vaginalis and the consequent shortening of the gubernaculum. Since the pars mesorchica joins the testis and epididymis, and since the latter is directly attached to the pars inguinalis, the combined effect of the ventral migration of the saccus vaginalis and the shortening of the gubernaculum is to bring the testis into the scrotal sac. This is attained by day 77. Although the testes respond to gonadotropins by day 70, there is evidence from studies on the prostate, Cowper's gland, and the pituitary gland that normally testis secretion does not begin prior to day 100. Therefore testicular hormone cannot be a factor in the major phases of descent.

6. The occasional melanophores which appear first in the connective tissue of the parietal tunica vaginalis, chorda gubernaculi, and ligamentum scroti of the 15-day male rapidly increase in number until the gubernaculum becomes black. These pigment cells are carried to the walls of the saccus

vaginalis with the reflection of gubernacular tissue into the parietal tunica vaginalis. Thus the testis comes to lie in a heavily pigmented sheath. In pouch young the density of melanophores at the gubernacular-tunica vaginalis junction is increased by administration of androgens and estrogens. The proportion of completely black cells increases while the proportion of greatly extended cells decreases with hormone treatment.

7. Melanophores make their first appearance in the round-body of the female at an age of 13 to 16 days. Their maximum number is attained between days 70 and 100 and is maintained thereafter. Androgenic and estrogenic treatments of pouch young increase melanophore number; the round-body is considerably hypertrophied by the injection of estrogens. Ovariectomized animals autopsied at day 83 or later possess less than half as many melanophores as normal controls, and their round-bodies are significantly smaller than normal. These results suggest that ovarian hormones begin to maintain the round-body and its melanophores before day 83.

The ground squirrel scrotal skin

1. The scrotal color of reproductively active ground squirrels is due to the heavy pigmentation of melanophores which lie at the dermal-epidermal junction. Their processes extend into the epidermis and probably supply melanin granules to the epidermal cells.

2. The scrotal melanophores are under testicular control. Castration of reproductively active adults is quickly followed by pigment reduction; gonadotropic treatment of intact immature males and androgenic treatment of intact or castrate immature males induce scrotal pigmentation.

3. The melanophores of the general integument of adult animals do not differ morphologically from those of the scrotal region. However, they are considerably less numerous and do not respond to testis hormone to any marked degree, if at all.

4. The integumentary darkening which begins 3 days after birth and disappears only near the end of the first season is due to melanophores which are morphologically similar to those of the adult scrotum and general integument. The normal sequence of juvenile pigment regression is not modified by castration or by the administration of androgens and gonadotropins. There is a strong negative correlation between degree of pigmentation and body weight.

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PLATE 1

EXPLANATION OF FIGURES

8 Photomicrograph of melanophores of the parietal tunica vaginalis of an adult normal male opossum. $\times 518$.

9 and 10 Photomicrographs from serial sections of normal opossums. $\times 14$. CG, chorda gubernaculi; CGH, chorda gubernacular head; IC, inguinal crest; IF, inguinal fold; LS, ligamentum seroti; SV, saccus vaginalis; WD, Wolffian duct.

9 Gubernacular apparatus at day 1; diagnosed male.

10 Gubernacular apparatus at day 1; diagnosed female.

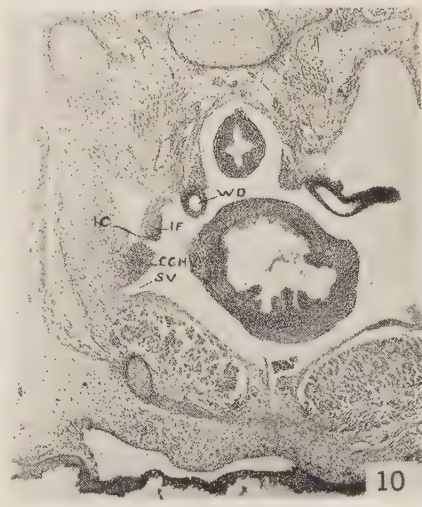
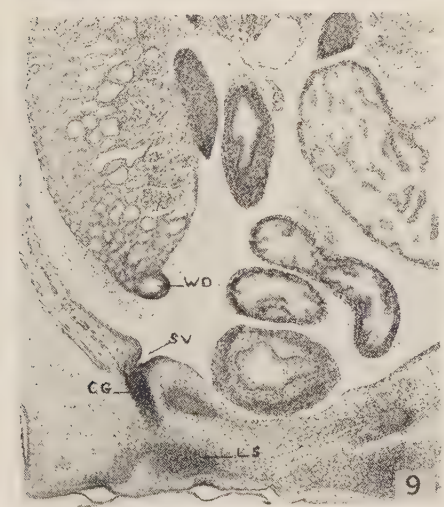


PLATE 2

EXPLANATION OF FIGURES

Photomicrographs from serial sections of normal opossums. $\times 14$. CG, chorda gubernaculi; IC, inguinal crest; IF, inguinal fold; LS, ligamentum seroti; ME, mesorchium; PI, pars inguinalis; RB, round-body; SV, saccus vaginalis; WD, Wolffian duct.

11 Gubernacular apparatus of male, day 8.

12 Deeply penetrated saccus vaginalis of male, day 31. Note numerous melanophores in the chorda gubernaculi; occasional pigment cells are seen in the parietal tunica vaginalis.

13 Section through caudal end of the testis of 52-day male. The densely pigmented ventral wall of the saccus vaginalis is derived from the gubernacular apparatus.

14 Gubernacular apparatus of female, day 7.

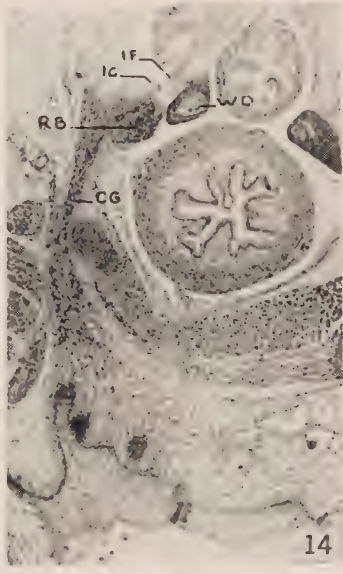


PLATE 3

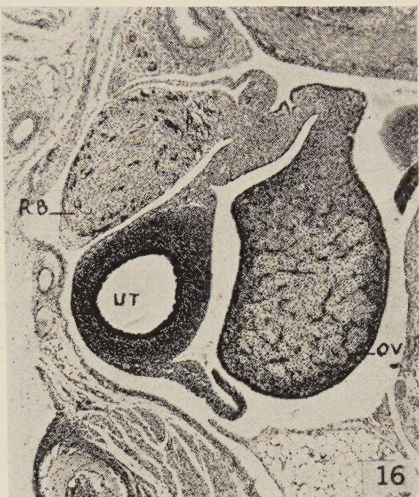
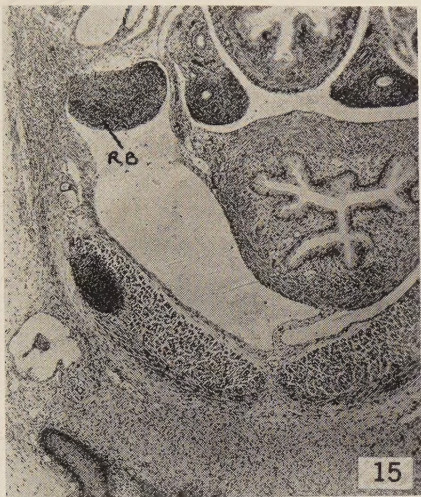
EXPLANATION OF FIGURES

15 and 16 Photomicrographs from sereal sections of normal opossums. $\times 14$.
OV, ovary; RB, round-body; UT, uterus.

15 Round-body of 12-day female.

16 Pigmented round-body of 44-day female.

17 Photomicrograph of the scrotal skin of a reproductively active adult normal male ground squirrel. Melanophores at the dermal-epidermal junction are seen sending their processes into the epidermis. $\times 1000$.



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